Exhibit 29

CHAPTER 24

Assessing Pelvic Epithelial Cancer Risk and Intercepting Early Malignancy

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Chapter Outline

Introduction

This chapter addresses the risk factors for pelvic (ovarian and fallopian tube) epithelial cancer, detection, and the role of the pathologist in risk reduction. The 5-year survival for stages IA and IV ovarian cancer are 88% and 18%, respectively, indicating that early detection may improve survival.[1] However, the opportunity to detect the tumors when they are limited to the ovary or fallopian tube may be difficult, because the period of time when these tumors are so localized is brief. Thus, with the exception of discovering novel therapies, reducing the death rate from pelvic epithelial cancer will involve (1) identification of women at high risk for pelvic cancers, (2) risk reduction, and (3) early detection of patients with cancer at lower and more curable stages.

Risk Identification

Genetic Ovarian Cancer Syndromes

There are three well-recognized genetic syndromes that account for the vast majority of familial ovarian cancer and approximately 10% of all ovarian cancers. These are breast ovarian cancer syndrome (BOCS), site-specific ovarian cancer syndrome (SSOCS), and hereditary non-polyposis colorectal cancer (HNPCC) syndrome (or Lynch syndrome).[2] Both BOCS and SSOCS are caused by inherited mutations in the *BRCA-1* and *BRCA-2* genes. In fact, although often described as separate entities, these two syndromes are most likely phenotypic variants of the same genetic mutations. *BRCA-1* and *BRCA-2* function as classic tumor suppressor genes and are inherited in an autosomal dominant fashion. Lynch syndrome is caused by mutations in DNA mismatch repair genes responsible for repairing errors in DNA replication. Inactivation of these genes result in a high incidence of right-sided colon cancer, endometrial cancer, and ovarian cancer.[3] Hereditary ovarian cancers associated with *BRCA-1* and *BRCA-2* mutations will be discussed first, followed by Lynch syndrome–related ovarian cancer.

Breast Ovarian Cancer Syndrome and Site-Specific Ovarian Cancer Syndrome

Germline mutations in the *BRCA-1* and *BRCA-2* tumor suppressor genes account for approximately 90% of cases of hereditary ovarian epithelial cancers and confer a risk of ovarian carcinoma by age 70 of 40% to 50% and 10% to 20%, respectively.[4-8] *BRCA-1* and *BRCA-2* are located on chromosomes 17q21 and 13q12-13, respectively, and are inherited in an autosomal dominant fashion. They encode nuclear proteins that are functionally similar. Both proteins participate in the repair of double-stranded DNA

Abstract

This chapter addresses several important aspects of ovarian cancer prevention. One pertains to the risk factors and efforts at early detection, critically reviewing the data on screening tests for ovarian cancer. Another addresses the role of the fallopian tube, a topic that has been at the center of efforts to reduce the risk of high-grade serous cancer via both risk reduction salpingo-oophorectomy and opportunistic salpingectomy. The role of the pathologist is first and foremost by more careful scrutinizing of the fallopian tube using the Sectioning and Extensively Examining the FIMbraited End (SEE-FIM) protocol, which has allowed all pathologists to participate in this paradigm shift to the oviduct as a potential source and target of prevention. Both supporting data and caveats are presented on this compelling issue that continues to evolve and the various entities that deserve attention as either potential premalignant lesions or their mimics are illustrated.

Keywords

BRCA
BOCS
hereditary ovarian cancer
serous tubal intraepithelial carcinoma (STIC)
risk-reducing salpingo-oophorectomy

damage, as well as the regulation of gene expression at a transcriptional level.[9-12] Loss of BRCA protein function leads to failure to repair DNA damage, resulting in the activation of p53, with subsequent initiation of cell cycle arrest or apoptosis. In the absence of functional p53, however, the cell continues to proliferate, DNA damage accumulates, and the likelihood of ensuing malignancy increases.

The lifetime risk of developing ovarian cancer in the United States is about 1.4%, but among women with *BRCA-1* and *BRCA-2* mutations, the risk has been estimated to be about 40% and 20%, respectively.[4,13,14] These genes also impart a significant lifetime risk of breast cancer in women and, in the case of *BRCA-2*, in men as well. Less than 0.3% of those in the general population are carriers of *BRCA-1* or *BRCA-2* mutations; however, the carrier rate is dependent on ethnic background.[7,15-17]

Founder mutations have been identified among multiple unrelated families in Iceland, the Netherlands, Sweden, and among Jews of Central or Eastern European (Ashkenazi) descent. The best-described founder mutations are the 185delAG and 5382insC mutations in *BRCA-1* and the 6174delT mutation in *BRCA-2*, occurring in Ashkenazi Jews at a carrier rate of 2%.[16] Although women of Ashkenazi Jewish descent do not have an overall increased rate of ovarian cancer, if an Ashkenazi Jewish woman develops ovarian cancer, it is far more likely to be genetic rather than sporadic. Consequently, if a woman of Ashkenazi Jewish descent develops ovarian cancer, there is a 40% chance she carries a mutation in one of these two genes.[18] The implications for her first-degree relatives (mother, sisters, daughters) are that they have a 20% risk for being gene carriers (given autosomal dominant transmission). Therefore, a woman of Ashkenazi Jewish heritage needs only one first-degree relative with ovarian cancer to be considered for further genetic counseling.[19] Routine screening of Ashkenazi women has been proposed given the high likelihood missing carriers based on family history alone.[20]

In contrast to hereditary breast cancers, in which *BRCA-1* and *BRCA-2* mutations are found with equal frequency, *BRCA-1* mutations are found more commonly than *BRCA-2* mutations in patients with familial ovarian carcinoma.[21-23] The mean age of developing ovarian cancer in the setting of a *BRCA-1* mutation is younger than in the women without a mutation (53 vs. 63 in the latter group).[24] Although *BRCA-1* carriers have a 2% to 3% risk of ovarian carcinoma by age 40, for *BRCA-2* carriers, this risk is not until age 50.[25] Mutations in *BRCA* genes are rare in the setting of sporadic ovarian tumors, but loss of function of their encoded proteins may play a role in tumor development.[26,27] Inactivation of *BRCA-1* in sporadic ovarian tumors has been attributed to promoter hypermethylation.[28,29]

The Society of Gynecologic Oncologists (SGO) has issued recommended criteria for referral of women to genetic counselors and consideration for genetic testing for *BRCA-1* and *BRCA-2* genes. These risk variables are summarized in Box 24.1. Because women with high-grade serous carcinoma probably have a higher rate of underlying germline mutations in *BRCA* (16% to 20%) than other ovarian tumors, testing should be considered for all women with this type of tumor.[30] Some have argued for population-based testing, independent of history, given the feasibility of such testing, risk associated with missing carriers, and variability in assessment of family history and referral to genetic counselors.[31] In addition, it is currently far more common to obtain expanded panel testing to include other lower penetrance genes.[32]

BOX 24.1 Society of Gynecologic Oncologists Guidelines for Identifying Women at Increased Risk for Having an Inherited Genetic Predisposition to Breast and Ovarian/Tubal/Peritoneal Cancer Who Should Receive Genetic Counseling and Be Offered Genetic Testing

Women *affected* with:
- High grade epithelial ovarian/tubal/peritoneal cancer
- Breast cancer ≤45 years old
- Breast cancer with a close relative[a] with breast cancer ≤50 years old or close relative[a] with epithelial ovarian/tubal/peritoneal cancer
- Breast cancer ≤50 years old with a limited family history[b]
- Breast cancer with two or more close relatives[a] with breast cancer at any age, with pancreatic cancer, or with aggressive prostate cancer (Gleason score ≥7)
- Two breast primaries, with the first diagnosed prior to age 50
- Triple negative breast cancer ≤60 years old
- With breast cancer and Ashkenazi Jewish ancestry
- Pancreatic cancer with two or more close relatives[a] with breast, ovarian/tubal/peritoneal, pancreatic, or aggressive prostate cancer (Gleason score ≥7)

Women *unaffected* with cancer, but with:
- A first degree or several close relatives[a] that meet one of the above criteria
- A close relative[a] carrying a known *BRCA-1* or *BRCA-2* mutation
- A close relative[a] with male breast cancer

[a]Close relative is defined as first-degree relative (parent, sibling, offspring), second-degree relative (grandparent, grandchild, uncle, aunt, nephew, niece, half-sibling), or third-degree relative (first cousin, great-grandparent, or great-grandchild).
[b]Limited family history includes less than two first- or second-degree female relative of female relatives surviving beyond 45 years old.
From Lancaster JM, Powell CB, Chen LM, et al: Society of Gynecologic Oncology statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol Oncol* 136(1):3-7, 2015.

Most ovarian malignancies diagnosed in women with *BRCA* mutations are high-grade serous carcinomas of advanced stage.[33,34] These tumors tend to have morphologic features known as *SET,* referring to more than 50% solid, pseudoendometrioid, or transitional.[35] Other histologic types have less frequently been described in patients with *BRCA-1* mutations, including endometrioid, clear cell, transitional, and undifferentiated carcinomas; carcinosarcoma; and dysgerminoma.[36,37] Borderline tumors and mucinous tumors do not appear to be associated with *BRCA* mutations.[38] The overwhelming majority of malignancies identified in the fallopian tube in women with *BRCA* mutations are also high-grade serous carcinomas.

Lynch Syndrome

Mutations in mismatch repair genes (Lynch syndrome) account for only a small percentage of hereditary ovarian cancer; these women have a lifetime risk of up to 12% for ovarian cancer.[39] Mutations in DNA mismatch repair genes *(MLH1, MSH2, MSH6,* and *PMS2)* most frequently lead to loss of function and, therefore, to microsatellite instability (MSI). Mismatch repair proteins function to

DNA base-pair mismatches. Microsatellites are repetitive DNA sequences that are prone to replication errors, such as base-pair mismatches. Loss of mismatch repair protein function results in an inability to repair these mismatches with resultant MSI. MSI is characterized by these repetitive DNA areas retracting or expanding and causing frameshift mutations. Tumors presumably form when the frameshift mutations occur within the coding region of genes involved in tumor development. Not all mismatch repair gene mutations bear an equivalent risk for ovarian cancer; mutations in *PMS2* are associated with the lowest overall risk.[40]

Most ovarian cancers in Lynch syndrome are non-serous histology, specifically endometrioid, clear cell, or undifferentiated carcinomas. Up to 10% of ovarian carcinomas in patients less than or equal to 50 years old are associated with Lynch syndrome.[41] There is a strong association between Lynch syndrome and clear cell carcinoma of the ovary, with mismatch repair protein mutations in 14% or 17% of clear cell carcinomas.[41,42]

Guidelines for Lynch syndrome in ovarian cancer are not well defined. The Amsterdam and Bethesda criteria primarily focus on colorectal carcinoma, but the SGO has also outlined guidelines for gynecologic tumors.[43] In general, testing for Lynch syndrome should be considered in woman with non-serous ovarian epithelial carcinoma. Because most women with Lynch syndrome who present with ovarian cancer are younger than 50 years old, many age-based algorithms must take this into account.[39] Molecular analysis of the DNA mismatch repair genes is the gold standard for definitive diagnosis of Lynch syndrome, but it is not routinely employed as a screening method due to cost and is used mostly for confirmation. Various algorithms for using mismatch repair protein immunohistochemistry, MSI analysis, and MLH1 methylation studies have been employed. In our institution, we screen all non-serous ovarian epithelial carcinomas by mismatch repair protein immunohistochemistry, with reflex to MLH1 methylation studies in cases where MLH1 expression is lost. This information is directly forwarded to our genetic counseling department to determine the need for further assessment and potential germline testing. Parenthetically, all women with ovarian cancers at the Dana Farber Cancer Institute/Brigham and Women's Hospital are referred for genetic counseling and undergo expanded panel testing if consenting. We no longer use family history to guide us.

Hereditary Predisposition to Ovarian Cancer: Beyond *BRCA* and Mismatch Repair Genes

Given that there are still families with strong histories of ovarian cancer with no identifiable mutations in *BRCA-1*, *BRCA-2*, or mismatch repair genes, current efforts are focused on identification of other inherited genetic mutations, which might account for the increased risk. Analysis of the large genomic library of single nucleotide repeats in large populations of cases and controls has been performed with the intent to identify loci that segregate with malignancy. This has been successful to a degree, identifying potentially predictive genomic markers for ovarian cancer. The downside has been the relatively low overall risk imposed by individual changes (less than twofold), which are too small to justify their use in a clinical setting, but combined risk models are emerging.[44-46] Multigene panel testing for cancer risk has become available to patients complicating management recommendations and further stressing the invaluable role of genetic counselors in the process. The risk associated with the genes is highly variable with about 20% of mutations considered to be variants of uncertain significance.[43,47,48]

Although *BRCA-1*, *BRCA-2*, and mismatch repair genes are certainly the most well-known and studied genes associated with a hereditary disposition to ovarian cancer, recent advances have identified other genes associated with potential increased risk.[43] Mutations in *BRIP1*, *RAD51C*, and *RAD51D* confer a lifetime risk of 10% to 15%.[49-51] A multi-gene profile (BROCA) is also in use.[52] *PALB2* gene mutations have been identified in some families with strong breast and ovarian cancer histories, but the actual risk of ovarian cancer with this gene is currently unknown.[49,53] Some recently identified mutations are associated with particular types of ovarian tumors. For example, *DICER1* mutations are associated with increased risk for the development of Sertoli-Leydig cell tumors, and *SMARCA4* gene mutations confer risk for ovarian small cell carcinoma.[54,55]

Demographic Risk Factors for Ovarian Cancer

Dietary Factors

Obesity has been reported to be associated with an increased risk of ovarian cancer mortality. There may also be an increased risk in women eating a diet high in saturated fat and low in vegetable fiber. In 1989, the observation that Swedes had both a high risk of ovarian cancer and the highest per capita dairy consumption in the world led some investigators to postulate a relationship between lactose consumption and ovarian cancer risk. Specifically, ovarian cancer cases were more likely to have high levels of galactose, a component sugar of the disaccharide lactose and a known oocyte toxin, than matched controls.[56] This observation, however, has been inconsistent. Overall, observational studies of diet and ovarian cancer risk have not shown a consistent pattern of association; therefore, no specific dietary strategy for ovarian cancer risk reduction can be recommended.[57-60]

Talc Exposure

Talc placed on the perineum may enter the vagina and ascend to the upper genital tract. Structurally similar to asbestos, there is theoretic concern that talc may potentially increase ovarian cancer risk. In addition, women who undergo tubal sterilization procedures or hysterectomy have a lower risk of ovarian cancer, supporting the ascending carcinogen hypothesis. Multiple case-control studies have shown a small but consistent increased risk with talc exposure, and a recent pooled analysis demonstrates a 24% increase in epithelial ovarian cancer (odds ratio [OR] = 1.24, 95% confidence interval [CI] = 1.15–1.33).[61] However, two prospective cohort studies found no overall increased risk for ovarian cancer associated with talc use.[62,63] Of note, one of those studies did show that there was a modest risk of the development of invasive serous carcinoma (relative risk

[RR] = 1.4, 95% CI = 1.02–1.09).[62] Overall, the evidence is mixed and inconclusive, with a possible risk associated with talc usage. Given the widespread availability and quality of cornstarch-based dusting powders and potential risk of talc-based powders, the practice of applying genuine talc to the perineum should be discouraged.

Infertility and Infertility Drugs

One of the most difficult issues to study is the association of infertility drugs and the risk of ovarian cancer. It is known, for example, that unexplained infertility is an independent risk factor for the development of ovarian cancer. One retrospective study claimed an association between prolonged clomiphene exposure and an increased risk of ovarian cancer. This study, however, was not restricted to invasive epithelial ovarian cancers but also included granulosa cell tumors.[64] These estrogen-secreting neoplasms of stromal origin may contribute to infertility directly by disrupting normal follicular maturation and the menstrual cycle. There are, however, a number of studies, including a large collaborative analysis of 12 case-control studies, that have reported an association between fertility drugs and invasive epithelial ovarian cancer.[65] In addition, many of the theoretic models of epithelial ovarian cancer pathogenesis implicate both incessant ovulation and high gonadotropin levels as important steps in malignant transformation of ovarian epithelium. Oral contraceptives that reduce ovulatory events and moderate gonadotropin levels are associated with a consistent and significant protective effect. It therefore seems prudent, in the absence of convincing data, to use fertility medication only when absolutely indicated, at the lowest effective dose, and for the shortest duration possible without compromising successful fertility treatment. Prior exposure to these agents should not be considered an indication for increased surveillance or prophylactic surgery.

Hormone Replacement Therapy

There appears to be an increased risk of ovarian cancer among women on estrogen replacement therapy (ERT). When compared with nonusers, users of ERT had a RR of ovarian cancer of 2.2 (95% CI = 1.53–3.17).[66] This risk increased with the duration of use. Long-term users, defined as at least 20 years of ERT use, had a RR of 3.2 (95% CI = 1.7–5.7).[67] Although some studies suggest a protective effect of combination replacement regimens including both estrogen and progesterone, this observation has not been confirmed. Based on these observations, long-term users of ERT should consider an increased risk of developing ovarian cancer as a factor in whether or not to initiate or continue ERT. Two recent meta-analyses suggested that women who use hormone therapy for 5 years from around 50 years old have about one extra ovarian cancer per 1000 users and, if its prognosis if typical, about one extra ovarian cancer death per 1700 users.[68,69]

Endometriosis

Endometriosis increased ovarian cancer risk with a RR of 1.3 to 1.8. Such cancers are more often low stage and of low-grade endometrioid and clear cell histology.[70] The risk is higher with increasing age and cyst complexity on ultrasound.[71]

Reducing Risk

Oral Contraceptives

Oral contraceptive pills (OCPs) significantly reduce the risk of developing ovarian cancer. A number of studies have demonstrated a 10% per year risk reduction up to 5 to 7 years of use.[72] This effect seems to persist for at least 10 years after OCPs are discontinued. This protective effect has also been observed in patients known to be carriers of the *BRCA-1* and *BRCA-2* genes and is the basis for recommending OCPs as a chemoprophylactic agent in known carriers who wish to retain their fertility.[73] There has recently been some controversy about the protective effects of OCPs in *BRCA* patients. An Israeli population-based study of OCPs and ovarian cancer demonstrated a protective effect of pregnancy but not of OCPs. It is unclear why the Israeli data are inconsistent with prior published reports.[74]

Tubal Ligation

Tubal ligation reduces risk by more than half and may be effective in subsets of women with *BRCA* mutations and family history of ovarian cancer.[75-77] The mechanism by which this procedure reduces risk is unknown, but the popular theory is that the transfer of growth factors or carcinogens is interrupted.

Risk Reduction Surgery

Women with inherited mutations in *BRCA-1* or *BRCA-2* genes carry a sizable risk of "pelvic" (ovarian/fallopian tube/peritoneal) cancer. This risk is attenuated as much as 80% to 90% in those who elect to undergo risk reduction surgery.[78,79] Following the increased attention to the fallopian tube as a source of high-grade serous cancer over the past 10 years, opportunistic salpingectomy has emerged as a recommended approach to reducing cancer risk in women undergoing sterilization or hysterectomy for benign disease.[80,81] This involves the removal of fallopian tubes for primary prevention of epithelial carcinoma of the fallopian tube, ovary, or peritoneum in a woman undergoing pelvic surgery for another indication. Based on retrospective population studies, the reduction in ovarian cancer risk is estimated at about 50% with a hazard ratio of 0.65.[82] Although not universal, this practice is being employed in many centers and more precise estimates as to the effectiveness of this technique should be forthcoming over the next one or two decades. Professional societies in several countries now recommend considering salpingectomy at the time of hysterectomy when convenient.[83] Opportunistic salpingectomy with temporary preservation of the ovaries is also being explored in women with *BRCA* mutations who refuse oophorectomy but there are no outcome data at present.[84,85] Table 24.1 provides a summary of the risk reduction for ovarian cancer provided by these potential preventative measures.

Table 24.1 Potential Preventive Measures for Ovarian Cancer

Method	Risk Reduction
Oral contraceptives	OR 0.11-0.80
Tubal ligation	OR 0.33-0.72
Prophylactic oophorectomy	HR 0.15
Salpingectomy	HR 0.65 (estimated 50% reduction)

HR, Hazard ratio; *OR*, odds ratio.
From Falconer H, Yin L, Grönberg H, et al: Ovarian cancer risk after salpingectomy: a nationwide population-based study. *J Natl Cancer Inst* 107(2), 2015; Yoon SH, Kim SN, Shim SH, et al: Bilateral salpingectomy can reduce the risk of ovarian cancer in the general population: a meta-analysis. *Eur J Cancer* 55:38-46, 2016.

Early Detection

There are two obvious theoretical reasons for ovarian cancer screening. The first is that tumors *arising in the ovary* begin as true stage I (A or B) tumors and early detection will predate de-differentiation or extra-ovarian spread. The second is that (at least theoretically) small neoplasms originating within the tubal mucosa or the ovary that are detected when the tumor burden is low would be more likely to be cured. Here it is important to separate early detection from the incidental removal of early malignancies during risk reduction surgery, which is not technically "early detection." By far, the role of risk reduction surgery, as implied previously, is to remove the tissue at risk rather than interrupt an evolving neoplasm. In one study, 5-year cancer-free rates for *BRCA-1* and *BRCA-2* mutation carriers were 96% and 69%, respectively, for those with prophylactic oophorectomy versus intensive surveillance. Moreover, even some of these patients with "early" disease have positive peritoneal fluid cytology, indicating early dissemination of malignant cells presumably came from occult tubal or ovarian primary neoplasms.[86,87] Nonetheless, when the early malignancy in the fallopian tube is confined to the mucosa (serous tubal intraepithelial carcinoma [STIC]) the risk of a subsequent pelvic serous cancer is approximately 5%, supporting the concept that these early lesions can be intercepted and cured.[88,89] Favorable short-term outcomes in small studies raise the prospect that sufficiently sensitive detection schemes could identify early disease and possibly enhance survival.[90]

Pelvic Examination

Pelvic examination is a central component of gynecologic care and permits the evaluation of potential abnormalities throughout the reproductive tract. However, the sensitivity of pelvic examination for the detection of ovarian cancer is only about 44%.[91] A 15-year study of pelvic examination alone uncovered six ovarian cancers in more than 18,000 examinations of 1319 women.[1,92]

Biomarker Screening Alone

Since the discovery of CA-125 in the 1980s, there has been little progress in the development of a molecular screening tool that promised to make a measurable impact on the death rate for ovarian epithelial cancer. The reasons for this are several:

- CA-125 is a high molecular weight glycoprotein that is elevated above 35 IU/mL in 85% of all epithelial carcinomas but in only 50% of women with stage I disease.[1]
- CA-125 elevations are associated with a range of other intra-abdominal disorders, cutting into the specificity of this marker.
- A test with 99% specificity will still necessitate as many as 25 invasive procedures to identify a single malignancy.[1] This level of precision has not been attained, even when multiple biomarkers have been used, including osteopontin and HE4.[65,93-96] One study proposed six biomarkers: leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, and CA-125, claiming both high sensitivity and specificity, but it was challenged as unduly optimistic.[97,98]

Another highly challenging variable in reducing the ovarian cancer death rate by conventional serologic screening lies in the nature of the disease itself.[99] One-fourth of ovarian cancers are associated with a pathogenesis that would favor screening—that is, tumors arising in the ovary that have a lag period prior to developing and metastasizing, specifically endometrioid, mucinous, and clear cell carcinomas. Clearly, these tumors would benefit from screening. Accordingly, when detected in stage I, they all have a good to excellent prognosis. Unfortunately, two-thirds or more ovarian cancers are high-grade serous (or high-grade endometrioid) and have spread to the pelvic organs by the time of diagnosis.[1] A recent report estimated the lead time that could be expected for serologic detection. Based on a longitudinal study with serologic biomarker analysis, the study concluded that levels of CA-125, HE4, and mesothelin began to rise as early as 3 years before the clinical presentation with malignancy; however, significant elevations were not seen until the last 12 months before presentation.[100] The efforts to develop diagnostic biomarkers the past two decades have come face to face with the stark implications of this reality if not the profound significance of understanding the origins and precursors of ovarian cancer. At present, the failure of biomarker detection to alter the disease has resulted in recommendations by every society and task force that routine screening for ovarian cancer not be performed.

Combining Biomarkers With Ultrasound and Other Imaging Techniques

There is evidence that transvaginal sonography (TVS) improves early detection of ovarian cancer and possibly influences mortality in some instances, but at a significant trade-off in specificity (Fig. 24.1).[101-103] Van Nagell et al. scored ovaries as abnormal if they exceeded 10 and 20 cm^3 from post- and premenopausal women, respectively. In a study of 8500 women who underwent TVS, 121 were abnormal and underwent surgery. A total of 57 had serous cystadenomas, and eight had ovarian cancers, six of which were stage IA. Only one each had an enlarged ovary by palpation or an elevated CA-125.[98] The implication from studies of this type is that TVS will downstage a proportion

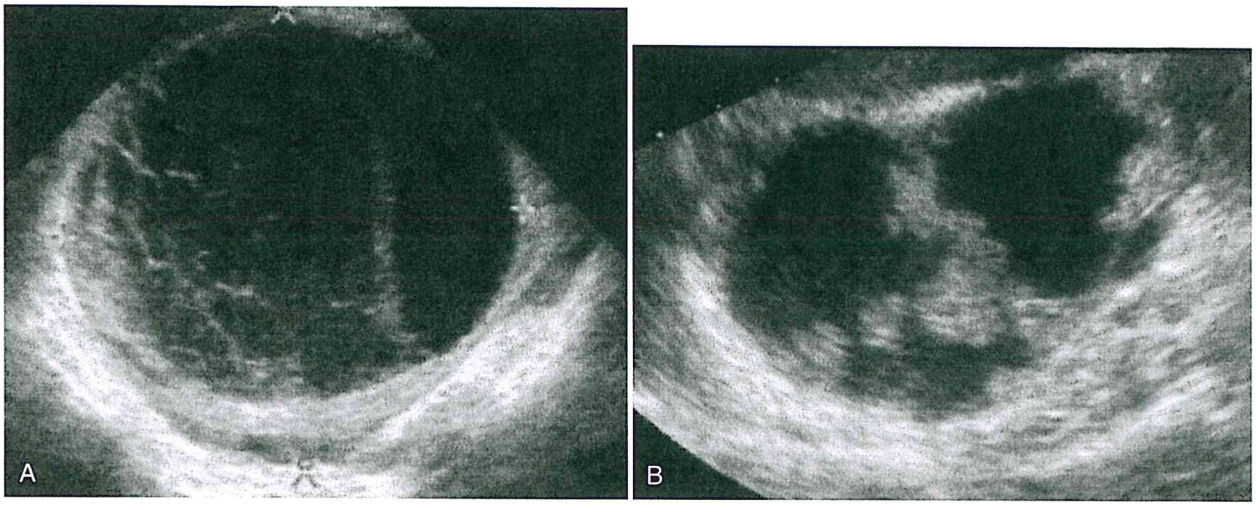

Fig. 24.1. Ultrasound evaluation of benign and malignant ovarian cysts. A, Hemorrhagic smooth-walled cyst. B, Ovarian cancer with irregular wall thickness, lining surface, and septation. (Courtesy of Beryl Bennaceraf, Boston, MA.)

of tumors and permit improved survival.[104] Despite these results, this group found that TVS had a sensitivity of 98.7% but a positive predictive value (PPV) of only 6.8%. Partridge and Barnes summarized five studies of 11,283 women, noting a PPV of only 3.1% for ovarian cancer.[1] In a study of TVS encompassing 42,113 screening years, van Nagell et al. defined an ovarian volume exceeding 10 cm in postmenopausal women or 20 cm in premenopausal women, or papillary or complex architecture as abnormal. Some 17 of 180 patients with persisting TVS abnormalities who underwent exploratory laparoscopy or laparotomy had cancer, 11 of which were stage I. Eight of the 11 did not have a palpable mass. TVS screening in this setting had a PPV of 9.4% with a negative predictive value of 99.97%. When nonepithelial tumors were excluded, the survival of ovarian cancer patients in the annually screened population was 92.9% at 2 years and 83.6% at 5 years. The authors cautioned that although the improved detection and reduced mortality were associated with screening, this benefit did not apply to women whose cancers occurred in the setting of a normal ovarian volume.[99] Additional studies have echoed the low PPV (1% to 27%) and false positive/true positive ratios of over 30, while reporting a small number of tumors diagnosed at stage I.[105-110]

A recent report summarized *sequential screening* with a baseline and three follow-up CA-125 and TVS assessments.[109] The PPVs were 1.4% to 1.8%, respectively. The ratio of surgical procedures to cancers detected was 31:1 at the initial screening and 14:1 on the subsequent three testing events. Similar to the studies described, 67% to 83% were stage III or IV, respectively, when diagnosed. Most of the surgeries were prompted by a positive TVS event. An important caveat is that these studies targeted ovarian enlargement, a strategy that ignores the early phases of serous carcinogenesis in the distal fallopian tube, ovarian, and pelvic surfaces. The same limitations apply to the use of ultrasound and biomarkers in combination. The Prostate, Lung, Colorectal and Ovarian (PLCO) trial show that screening with both CA-125 and transvaginal ultrasound compared with standard care did not reduce ovarian cancer mortality.[111]

A recent trial garnering considerable attention was the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKTOCS) that compared yearly CA-125 (using an algorithm) and transvaginal ultrasound (multimodality screening [MMS]) to ultrasound alone and no screening. Over 200,000 women were enrolled. Although the primary analysis did not appear to show a reduction in mortality for MMS, there was a 20% mortality reduction at 7 to 14 years.[112] The authors concluded that this was due to removal of prevalent cases (years 1 to 7). Caveats include the following:

- The notion that the differences in mortality between early and later years of the study are due to prevalent versus incident disease is unproven.
- Estimates of ovarian cancer mortality were based on death certificate data in the compared populations, and the precise reason for reduced mortality estimates is not known.
- There is no evidence that this screening algorithm will alter mortality in women with *BRCA-1* or *BRCA-2* mutations, where the ovarian cancer outcome estimation would be far less prone to error.

This management algorithm is currently not recommended by the U.S. Food and Drug Administration (FDA). Various other algorithms which combine imaging, biomarkers, and clinical signs have been proposed but have not yet been widely adopted.[113,114]

Molecular Screening for Ovarian Cancer

With the emergence of highly sensitive molecular assays for gene mutations, a few studies have explored the possibility that ovarian or tubal cancers could be detected via analysis of lower genital tract fluids. In theory, these "molecular Papanicolaou (Pap) tests" would detect mutations by sequence analysis targeting common mutations

associated with gynecologic cancers. Kinde et al. successfully identified mutations in liquid-based Pap tests from 9 of 22 ovarian carcinomas.[115] It remains to be seen whether this approach will detect early ovarian or tubal carcinomas in their curable stages or will encounter problems with "background mutations" in the genital tract mucosa of healthy women.

Presenting Signs and Symptoms

Clinical presentations with borderline early and advanced cancer vary. Webb et al. examined patients in these groups and noted 16%, 7%, and 4% of patients with borderline disease, early ovarian cancer, and advanced ovarian cancer, respectively, were symptom free. Abdominal pain (44%) and swelling (39%) were the most common symptoms as opposed to abdominal mass and gynecologic symptoms (12% each). Gastrointestinal problems and malaise were more common in advanced disease.[116]

Overall, ovarian volume is expected to decrease with age.[117] In patients with a suspected pelvic mass, TVS, with or without CA-125 assessments, is of considerable value in segregating low- versus high-risk patients and can be used as a guide to intervention (see Fig. 24.1).[1,118] The presence of a mass with conspicuous calcium supports the diagnosis of teratoma. In contrast, any solid tumor is of concern. Numerous investigators have proposed scoring systems, which depend on specific characteristics of tumors that predict risk. Certain groups can be identified with a strikingly low risk. Bailey et al. showed that none of unilocular cysts less than 10 cm in diameter proved to be malignant.[119] Half resolved spontaneously; many of the rest were cystadenomas. In contrast, complex cysts had a similar resolution rate, but seven of 114 proved to be malignant.[74] In a study of more than 3200 unilocular cysts, nearly 70% resolved spontaneously. Of the remainder, 16.5% (of the total) developed septation, 5.8% a solid area, and 6.8% persisted as a unilocular lesion. Of 27 cancers emerging in the persistent group, 10 were associated with the unilocular lesions, underscoring the need to follow all patients with a persistent ovarian lesion.[120]

The Role of the Pathologist in Risk Reduction and Early Detection

There are two opportunities for the pathologist to participate in the early detection of ovarian cancer:

- Routine analysis of the distal fallopian tube in women undergoing procedure for benign disorders: The routine analysis of the distal fallopian tube in salpingectomies from benign disorders will occasionally uncover an early (intraepithelial) carcinoma in that site. Several studies of limited populations employing comprehensive examination of the fallopian tube similar to the Sectioning and Extensively Examining the FIMbraited End (SEE-FIM) protocol have estimated the frequency of occult serous carcinoma to be between 0.6% and 1.1% in women with no history of or risk factors for serous carcinomas of the uterus or other pelvic sites.[121,122] In a recent report from Brigham and Women's Hospital covering 10 years, the frequency of tubal serous carcinoma was estimated to be between 0.1% and 0.2%.[123] Notably, many of the early carcinomas were associated with concurrent endometrioid carcinomas of the uterus; the significance of this association (which is still uncommon) is under study.
  The detection of these rare, but early cancers will permit both a thorough workup of the patient for metastatic disease and, in the right setting, a rationale for genetic testing, which if positive could benefit others in the family.[124,125] It is our practice to submit the fimbriated end of the fallopian tube in addition to representative cross-sections in these cases.
- Systematic analysis of fallopian tubes from women who are deemed at risk for ovarian cancer (i.e., those with known inherited mutations in the *BRCA-1* or *BRCA-2* gene): The more conventional exercise is the evaluation of the prophylactic salpingo-oophorectomy from these women for evidence of occult malignancy. However, in recent years, a proportion of these tumors have been found in the fallopian tube mucosa, leading investigators to propose a tubal origin in many instances. Moreover, the frequent location of these early lesions in the fimbriated portion of the tube mandates thorough attention to this portion of the structure. This discussion will address the following points: (1) the beneficial role of prophylactic surgery, (2) the risk of malignancy in women with mutations in *BRCA*, (3) the procedure for evaluation of the fallopian tube, (4) the criteria for tubal intraepithelial carcinoma, (5) the pitfalls in the diagnosis of early tubal malignancies, and (6) the potential impact on clinical management and perceptions of serous carcinogenesis.

Prophylactic Surgery and Detection of Early Malignancy

Bilateral salpingo-oophorectomy in women with *BRCA* mutations reduces the risk of developing pelvic serous cancers by greater than 90%.[78,126] Laparoscopy (whenever possible this is the preferred modality) and laparotomy (almost never) are the surgical modalities of choice to allow inspection of the peritoneal surfaces at the time of prophylactic salpingo-oophorectomy and collect fluid for cytologic evaluation. Performing a hysterectomy in addition to removing the adnexa does not appear to improve the efficacy of the procedure. Paradoxically, women with *BRCA*-associated cancers have a better clinical outcome following treatment than those without *BRCA* mutations, even though the former tend to present at a higher stage. Presumably, this is due in part to *BRCA*-associated tumors being more susceptible to chemotherapeutic agents, such as cisplatin, that induce double-stranded DNA damage.[127]

Prophylactic surgery may reduce, but not completely eliminate, the risk of ovarian cancer in high-risk individuals. Bilateral salpingo-oophorectomy in *BRCA* carriers reduces ovarian cancer risk by over 90% and breast cancer risk by over 50%.[29,124] The operation should be reserved for women with known mutations in *BRCA-1* or *BRCA-2* (or other lower penetrance genes with deleterious mutations) or those who have a family history consistent with one of the genetic syndromes associated with ovarian cancer, and

it should include an evaluation by a genetic counselor.[9] The addition of hysterectomy does not appear to increase the efficacy of the operation and should be performed only for concurrent gynecologic indications or if the patient has HNPCC. Patients should be informed that prophylactic surgery does not protect them against the subsequent development of high-grade serous carcinoma of the peritoneum. They should also be warned that about 7% of prophylactic operations detect occult ovarian or tubal carcinoma and that these lesions may not be appreciated until final pathology reports are available.[128] Pathologists should be instructed to submit the entire specimen for sectioning to reduce the risk of missing a microscopic occult malignancy. Finally, the patient should be prepared for the consequences of surgical menopause.

An additional risk that has been addressed recently is that of a subsequent uterine serous adenocarcinoma. One study computed an odds ratio of 22 for women with *BRCA-1* and 6.4 for those with *BRCA-2* germ line mutations. These estimates were based on a very small number of cancer cases across multiple institutions; however, three of three did show loss of wild type *BRCA-1* gene.[129] Thus this risk, albeit small, will enter into the discussion when counseling women with these mutations.

In studies of women undergoing prophylactic salpingo-oophorectomy for *BRCA-1* or *BRCA-2* mutations *(BRCA+)*, the risk of uncovering an early cancer has been estimated to be about 5% to 10%, a figure that may vary both as a function of the age of the population and the thoroughness with which the specimen is examined.[90,130-139] Many of these early cancers have been identified in the distal fallopian tube, the earliest malignancy being a tubal intraepithelial carcinoma. In a review of nearly 20 cases in our institution, the frequency of a tubal intraepithelial carcinoma was over 80%.[140] In the largest study to date (GOG-0199), clinically occult cancers were detected in 15 of 326 (4.6%) *BRCA-1* carriers and 8 of 231 (3.5%) *BRCA-2* carriers undergoing prophylactic salpingo-oophorectomy; about half of these tumors were more than stage II.[141] With these figures in mind, the following points must be stressed:

- Early malignancies can be both serous (STIC, 80%) and rarely, endometrioid (ETIC, 20%).[130,134]
- Twenty percent of these early serous cancers are not associated with an intraepithelial lesion in the fallopian tube; more extensive tubal sampling might uncover some, but most of the time it will not.[142]
- Advanced pelvic serous cancers in *BRCA+* women are virtually identical in tumor distribution to the general population with this disease (i.e., less than half will have evidence of a tubal origin).[130,143] Although it may be tempting to attribute this to obliteration of the tubal mucosal origin by expanding tumor or some other sampling artifact, *the reader is strongly cautioned that salpingectomy alone may not rid the patient of risk, and leaving the ovary might even place her at a substantial risk of an eventual pelvic serous cancer.*
- Prophylactic specimens from women with a family history of cancer but without a documented *BRCA* mutation are unlikely to contain an early malignancy.[144] Virtually all genetically related pelvic serous cancers are linked to *BRCA+*.
- As mentioned earlier, STICs are also rarely reported in fallopian tubes removed as part of surgery for indications other than serous carcinoma.[118,121-123,145]

The SEE-FIM Protocol

Prophylactic salpingo-oophorectomies from women with mutations in *BRCA* or strong family histories of ovarian carcinoma should be submitted entirely and examined histologically. Most of the early cancers described in the preceding sections occur in grossly unremarkable specimen, so it is imperative that all the tissue be evaluated in order to properly treat the individual and to further understand the pathogenesis of this disease.

The fallopian tubes and ovaries should be submitted entirely and be evaluated in serial sections by a pathologist with expertise in gynecologic malignancies. At Brigham and Women's Hospital, the goal is to ensure sectioning and extensive examination of the fimbria (SEE-FIM protocol), because the majority of early serous tumors occur in this area, which is as follows (Fig. 24.2):

- The entire tube is initially fixed for at least 4 hours to minimize loss of epithelium during manipulation.
- The distal 2 cm of the fimbriated end is amputated.
- The fimbrial mucosa is sectioned longitudinally into four pieces. The remainder of the tube is sectioned transversely every 2 to 3 mm into cross sections.
- The tubal segments combined with the fully sectioned fimbriated end are submitted in toto.[146]

Recent studies show increased rates of detection (from less than 2.5% to 17%) with more thorough sectioning.[127] Thus, protocols for examining the fallopian tubes in genetically susceptible patients will continue to evolve. We have used a protocol whereby the laboratory makes several sections from each fimbrial block. However, a more economical and probably equally effective method is to section the fimbria as finely as possible, maximizing the surface area that is examined, and examine a single section. If an area of concern is encountered, it can be evaluated by further serial sections.

Fig. 24.2. Sectioning and Extensively Examining the FIMbraited End (SEE-FIM) protocol for tubal examination. One tube is shown here. The fimbria *(lower row)* is finely sectioned in sagittal orientation. The remainder of the tube *(upper row)* is sectioned at 1- to 2-mm intervals. (Photo courtesy of Dr. Eric Huang.)

Histologic Criteria for Early Tubal Cancer (Serous Tubal Intraepithelial Carcinoma)

As a consequence of the increasing surgical practice of prophylactic salpingo-oophorectomy for women with *BRCA* mutations, more and more serous carcinomas are being discovered in the fallopian tube and at an early stage.[89] This will eventually alter the currently perceived ratio of fallopian tube/ovarian carcinomas (0.41 and 15 per 100,000/year).[147,148] In applying histologic criteria, the practitioner must be cognizant of the fact that terms used previously for early malignancies should be selected with caution, inasmuch as the literature is replete with terms such as dysplasia or atypical hyperplasia.[146,149] Such terms may appropriately identify epithelial changes of uncertain biologic significance, but they cannot be used without carefully qualifying their risk to the patient. We resort to a single term *serous tubal intraepithelial carcinoma (STIC)*, because most of the lesions are serous-type.

STICs can be found in both prophylactic salpingo-oophorectomies, in the tubes of women with pelvic serous carcinoma,[88,90,146] and, rarely, in routine salpingo-oophorectomy specimens.[118,142,150] The presence of STIC implies an origin in the distal fallopian tube, although this point remains controversial, inasmuch as an alternative explanation would be spread from an ovarian or peritoneal primary to the endosalpinx. We do not classify a lesion as an STIC unless there is clear association with normal mucosa without underlying malignancy, including vascular space invasion. Either could give rise to intramucosal tumor. Overall, intramucosal neoplasia manifesting as spread to the tube from another site is uncommon but is being recognized more in studies that pay close attention to the tubes in intra-abdominal malignancies.[151]

The following features characterize a tubal intraepithelial carcinoma (Table 24.2 and Figs. 24.3 and 24.4):

- *Most but not all exhibit epithelial stratification, but virtually all should exhibit some degree of depolarization,* often with exfoliation of cells into the lumen. The lesion at low power may assume a slightly thickened, almost velvety appearance, with a slightly irregular surface relative to the adjacent non-neoplastic mucosa. Intraepithelial fracture lines may appear as a function of disaggregation of the stratified epithelial cells.
- The neoplastic cells can be variable in size but are often noteworthy for their uniformity, owing to the lack of the usual heterogeneity seen with a mixed ciliated and secretory cell population. The high nuclear/cytoplasmic (N/C) ratio gives them a somewhat more basophilic appearance as well. A mildly stratified monomorphic population can often be easily appreciated at low magnification.
- Nuclear features vary, but rounded enlarged nuclei with prominent nucleoli are common. Marked nuclear

Table 24.2 Assessment of Serous Tubal Epithelial Proliferations

Parameter	Benign Serous Tubal Epithelial Proliferation (p53 Signature)	Serous Tubal Epithelial Proliferation of Uncertain Significance	Serous Tubal Intraepithelial Carcinoma
N/C ratio	Variable	Variable	High
Thickness	Variable	Variable	High
Nucleoli	Occasional	Common	Common
Molding	Occasional	Variable	Variable
Cell shape	Round/oblong	Round/oblong	Round
Unpolarized	No	No	Yes
Exfoliation	No	No	Common
Intraepithelial fractures	No	No	Common
p53 staining abnormality[a]	Yes	Yes	Yes
Cyclin E	Negative or patchy	Variable	Usually strong[c]
p16	Negative or patchy	Variable	Usually strong[c]
MIB-1 (index in the most proliferative area)[b]	<20%	20% to 50%	40% to 90%

N/C, Nuclear/cytoplasmic.

[a]A subset of serous tubal intraepithelial carcinomas (STICs) will stain completely negative because of a deletion mutation in the sequence encoding the antigenic target.

[b]Approximate proliferation index ranges are given. Proliferation index is not a primary factor in lesion classification.

[c]Expression of some genes (ALDH-1, PAX-2, Cyclin E, p16) can change abruptly. Thus no biomarker is completely reliable at this point in identifying STIC.

From Mehra K, Mehrad M, Ning G, et al: STICs, SCOUTs and p53 signatures; a new language for pelvic serous carcinogenesis. *Front Biosci (Elite Ed)* 3:625-634, 2011; Chen EY, Mehra K, Mehrad M, et al: Secretory cell outgrowth, PAX2 and serous carcinogenesis in the Fallopian tube. *J Pathol* 222:110-116, 2010; Roh MH, Yassin Y, Miron A, et al: High-grade fimbrial-ovarian carcinomas are unified by altered p53, PTEN and PAX2 expression. *Mod Pathol* 23:1316-1324, 2010; Ning G, Xian W, Crum CP: Unpublished data.

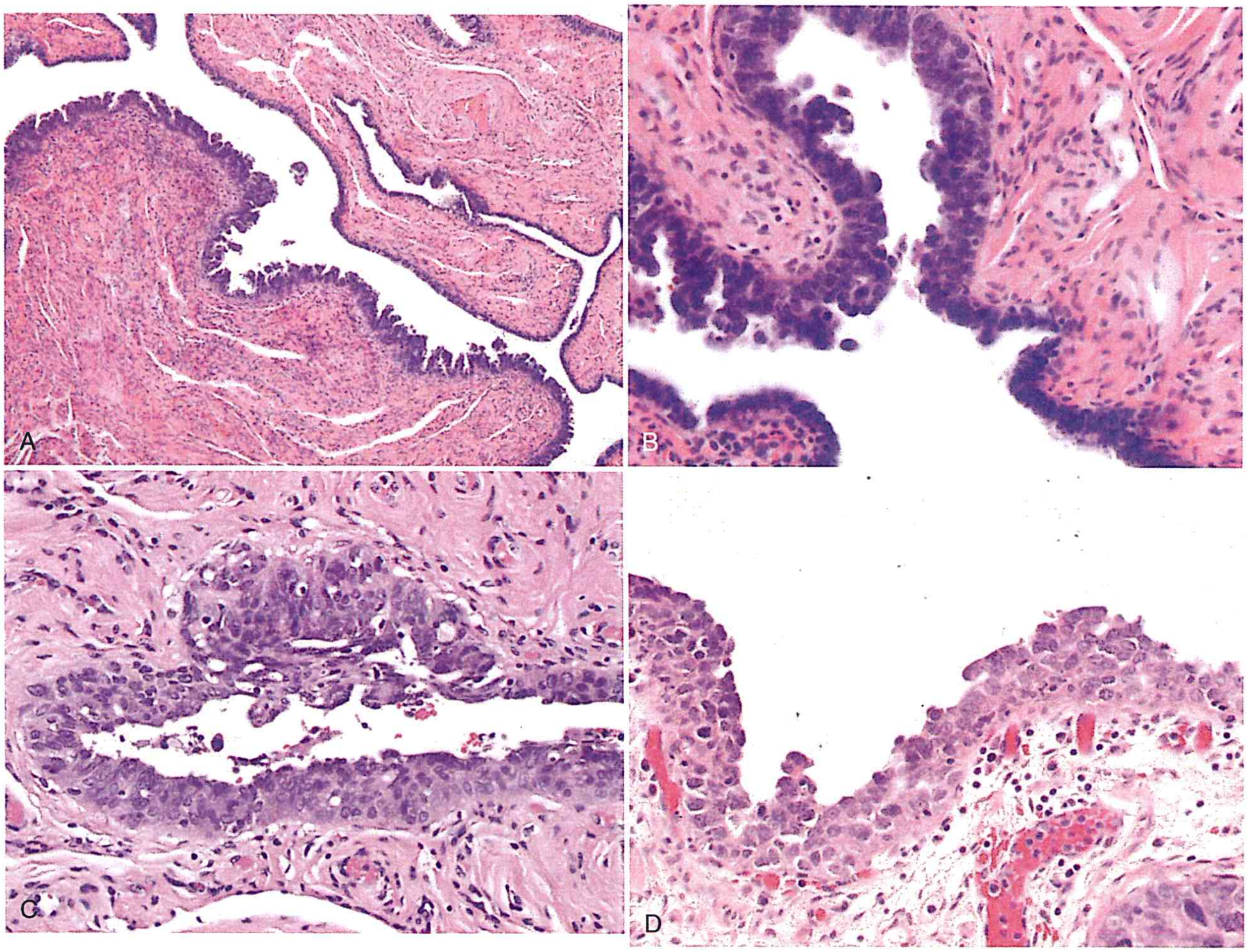

Fig. 24.3. A, Low-power image of a thickened neoplastic epithelium in the fimbria. B, At higher magnification, note the stratification and fracture lines, with exfoliation. C, Another serous tubal intraepithelial carcinoma (STIC) with marked loss of polarity, irregular thickness, and horizontal fracture lines. D, This STIC is slightly more deceptive, with a lower nuclear/cytoplasmic (N/C) ratio. However, note the loss of polarity and exfoliating neoplastic cells on the surface.

variation is the exception rather than the rule. Nuclear molding is another feature, but in our experience, is not reliable by itself. *Of note, foci containing nuclear enlargement, nucleoli, and molding can be observed in otherwise benign-appearing epithelium.*

- STICs, specifically those associated with serous carcinomas, are usually diffusely positive for p53 and p16 (see Fig. 24.4). A minority are completely p53-negative due to deletion mutation of the gene that prevents recognition of the protein by the antibody (Fig. 24.5). Thus a completely p53-negative stain in the area of interest does not exclude tubal intraepithelial carcinoma. p16 may be helpful, although we have seen p16 staining in some nonmalignant tubal epithelia. Cyclin E is also upregulated in tubal intraepithelial carcinomas but can be seen in nonmalignant epithelia. Novak et al. reported a strong correlation between concurrent staining for both Stathmin1 and p16ink4 and STIC.[152] This combination may be helpful, although like most biomarkers, staining is not invariably predictive of STIC.
- The proliferative (MIB-1) index is typically high, exceeding 70% in at least a portion of the lesion. However, it may vary considerably, particularly in endometrioid STICs.
- The diagnosis of STIC should be made morphologically, with stains used to enhance and refine the histologic findings. Corroboration of the diagnosis by another pathologist is prudent, particularly if there is any uncertainty. The reproducibility of diagnosing STICs has been only fair to good, but a recent algorithmic approach has improved this dramatically.[153-155]

Endometrioid Tubal Intraepithelial Carcinoma

Although more unusual than STICs, endometrioid intraepithelial carcinomas are sometimes found in the fallopian tube epithelium. These lesions demonstrate stratified columnar or cuboidal epithelial cells with an endometrioid appearance (Fig. 24.6). The N/C ratio is lower than in STICs. They are typically negative or weakly positive for p53 and demonstrate a variable MIB-1 index.

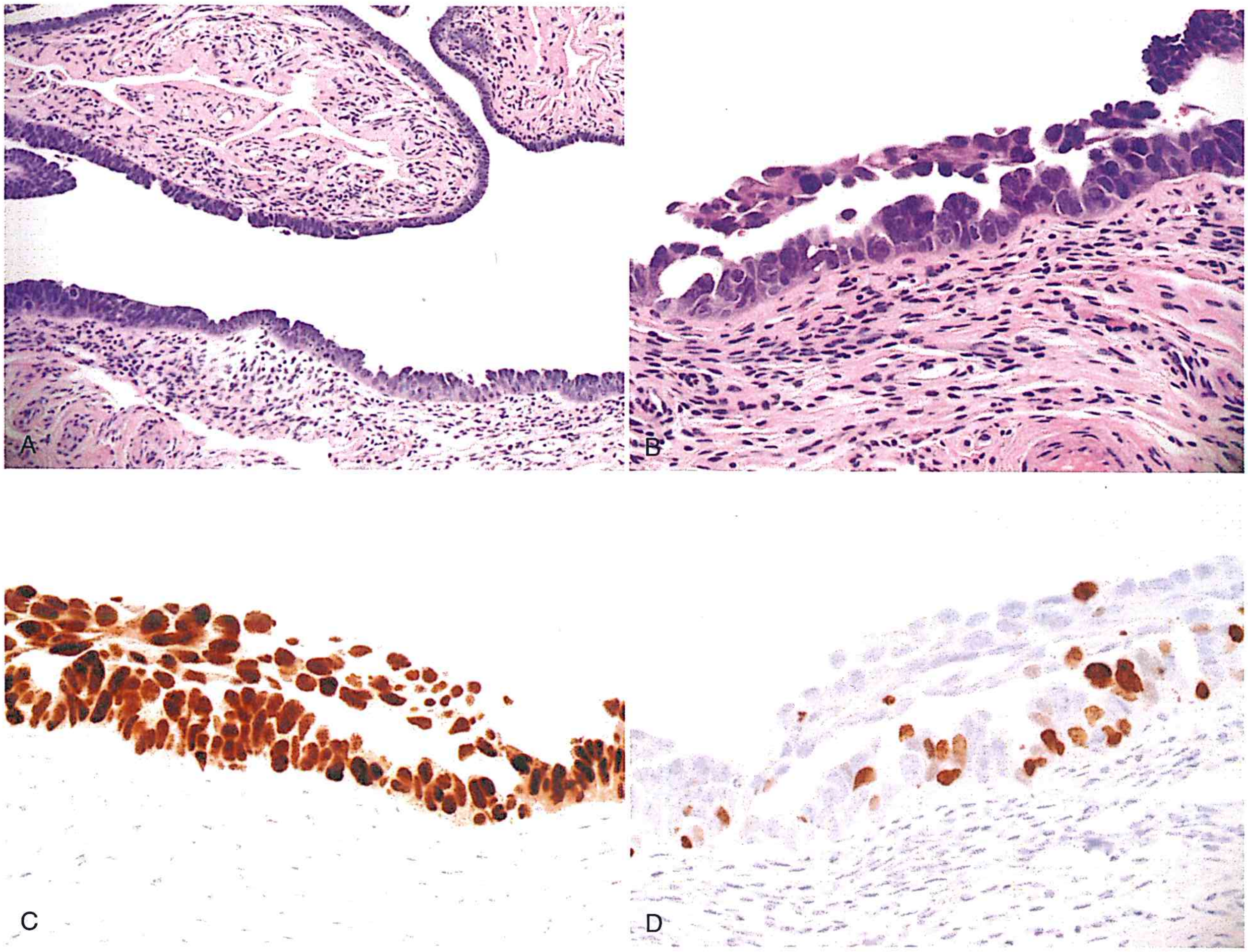

Fig. 24.4. A and B, A serous tubal intraepithelial carcinoma (STIC) exhibits (C) strong positivity for p53 and (D) a high MIB-1 index.

Mucinous Tubal Intraepithelial Carcinoma

We have rarely encountered mucinous intraepithelial neoplasia in the distal fallopian tube. It is included in this section for completeness and because we have no evidence to refute it as a primary lesion. An interesting feature of these mucinous tubal intraepithelial carcinomas is the not infrequent strong staining for both p16 and p53, suggesting that etiologically they are closer to STICs than endometrioid lesions (Fig. 24.7). Presumably they signify some element of plasticity in tubal secretory cell differentiation, akin to an intestinal type.

Early Invasive Serous Carcinoma

The point at which an STIC transits to an invasive carcinoma may be difficult to ascertain and may be moot (Fig. 24.8). Papillary architecture with marked complexity in epithelial growth may not be accompanied by invasion but almost certainly conveys a high risk of peritoneal disease. Likewise, if solid growth is present in the stroma, a diagnosis of invasion is made and may very well be followed by adjunctive chemotherapy. Because these lesions can be on the order of 1 or 2 mm in diameter, when an STIC is identified, it is important to perform serial sections in addition to immunohistochemical stains, because sectioning can sometimes reveal an underlying invasive lesion. The morphology of the cells is the same as that for the STIC, except that there is architectural evidence of invasion.

Differential Diagnosis of Serous Tubal Intraepithelial Carcinoma

The differential diagnosis of STIC or early malignancy includes a limited number of possibilities and must always be approached in the context of the clinical picture (see Table 24.2). A number of potentially confusing alterations, including complex transitional metaplasia, fimbrial adenofibromas, papillary hyperplasia, Arias-Stella effect, inflammatory atypias, and others, are discussed in a previous chapter. This discussion is confined to a spectrum of intraepithelial changes that are most often encountered in the distal fallopian tube, in increasing degree of atypia.

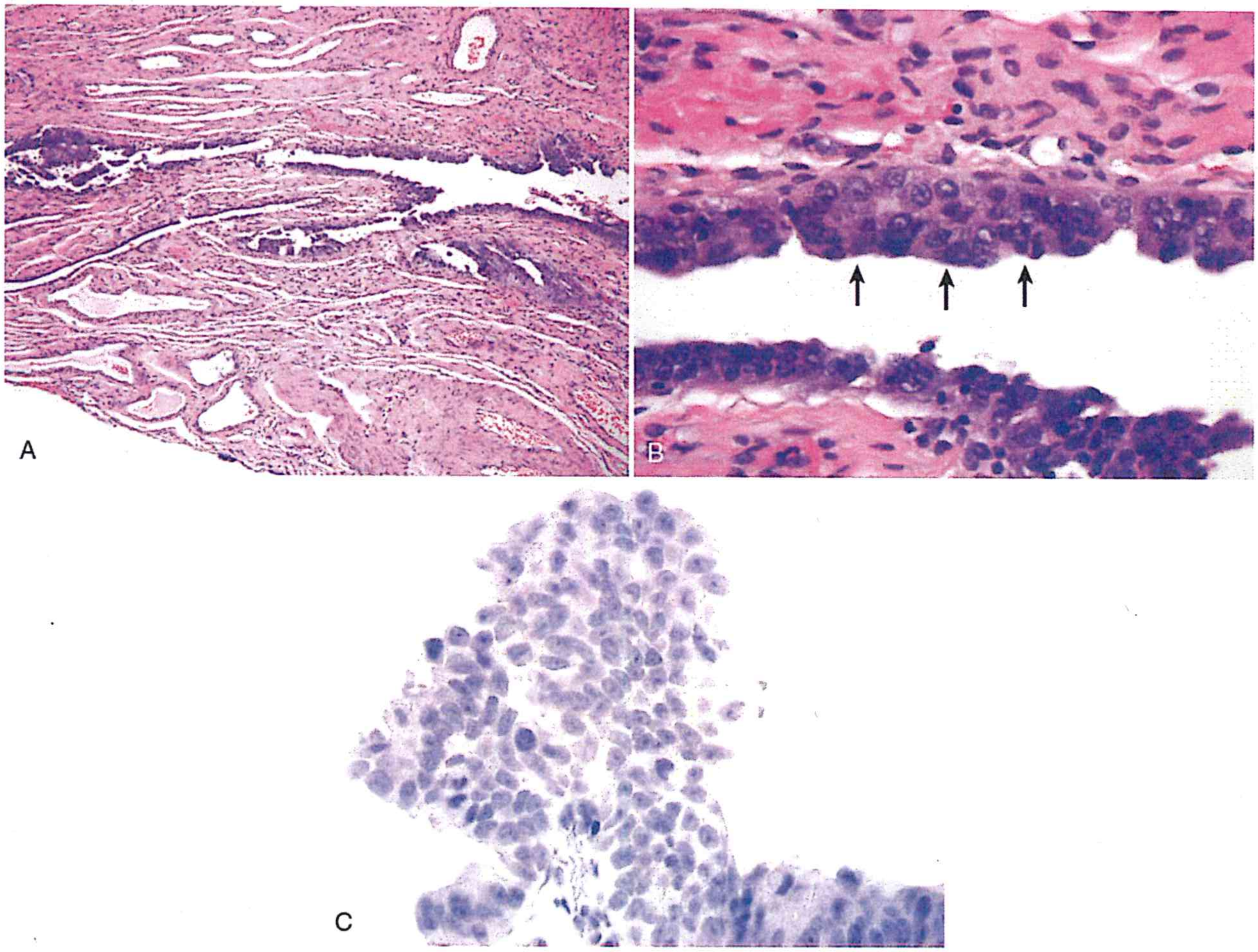

Fig. 24.5. Early tubal malignancy in a woman who underwent salpingo-oophorectomy for a benign ovarian fibroma. A, Low magnification highlights an irregular epithelial surface. B, Higher magnification. Note the rounded cells and loss of polarity with true stratification *(arrows)*. C, Complete absence of p53 immunostaining is characteristic of a deletion mutation in the gene.

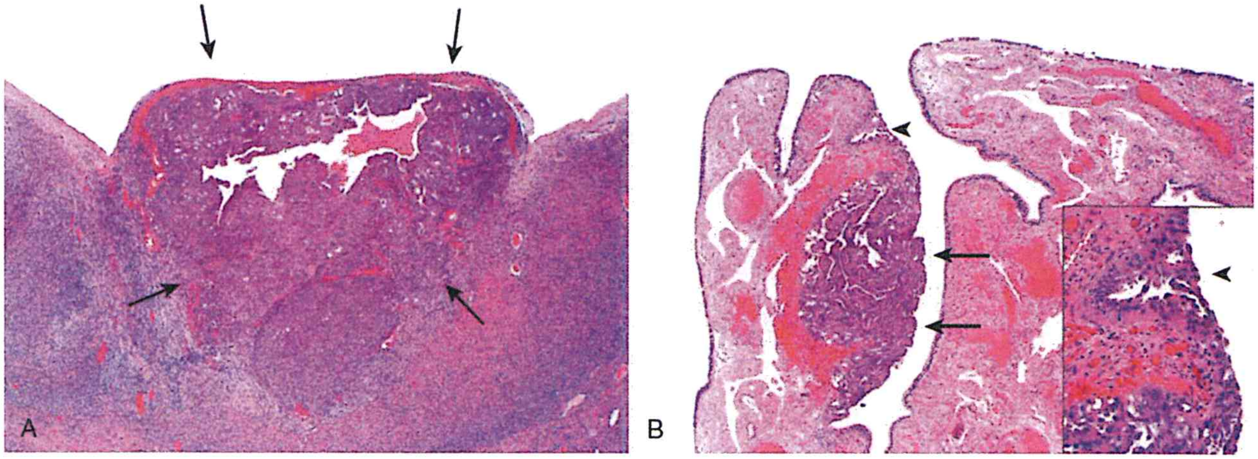

Fig. 24.6. Endometrioid carcinoma in a *BRCA+* individual. A, A focus of endometrioid carcinoma on the ovarian surface *(arrows)*. B, The distal tube contains a microscopic endometrioid tubal intraepithelial carcinoma *(arrows)*; *(inset)* at higher magnification *(arrowheads)*.

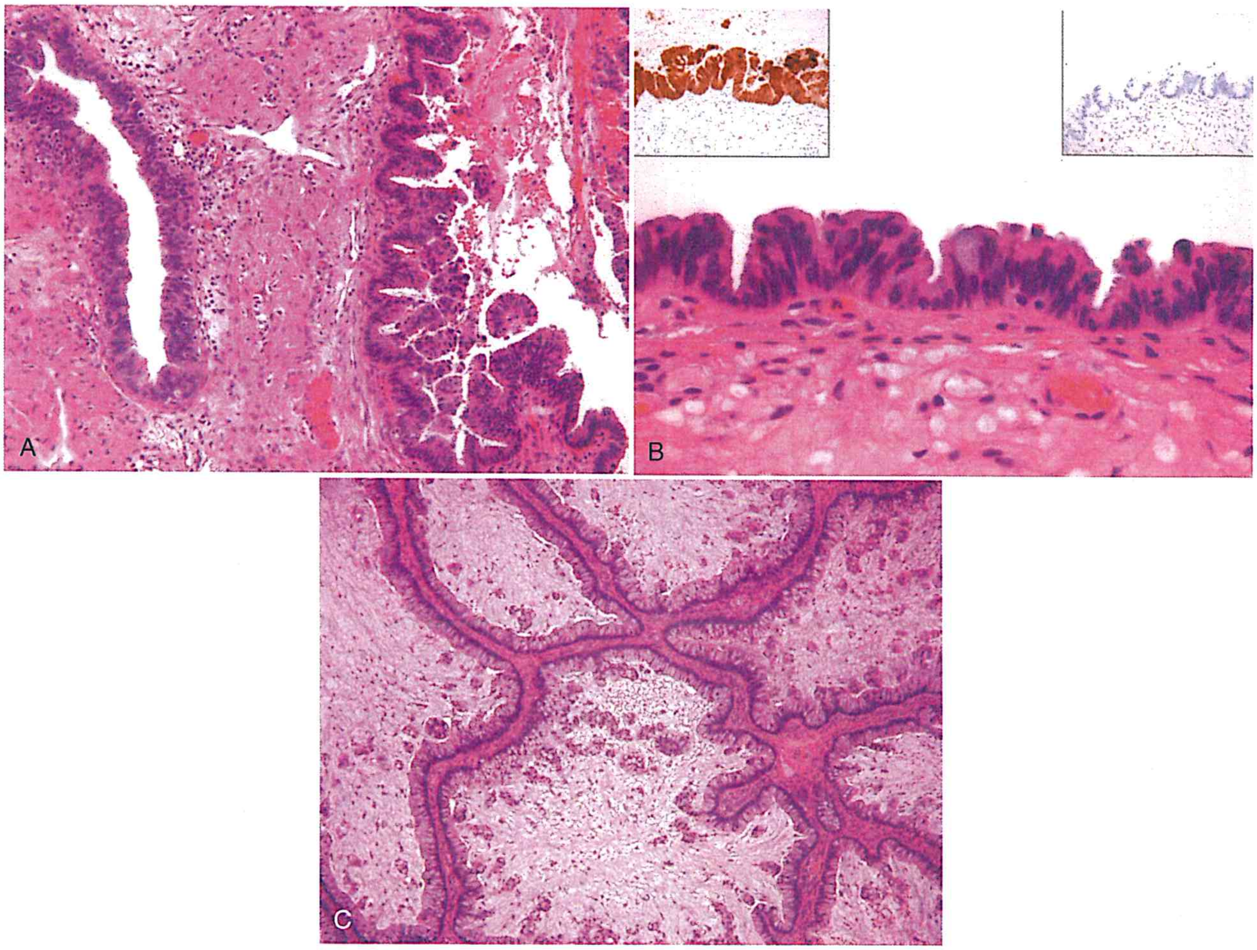

Fig. 24.7. **A,** Mucinous tubal intraepithelial carcinoma. **B,** At higher magnification with strong staining for p16 *(upper left inset)* and absence of p53 staining *(upper right inset)*. **C,** The adjacent ovary contained a mucinous carcinoma.

Benign Tubal Epithelial Hyperplasias (Secretory/Stem Cell Outgrowths)

Secretory/stem cell outgrowths (SCOUTs) are common findings in the tube and consist of what appear to be small clonal expansions of epithelial cells with variable ciliated cell differentiation. They do not harbor mutations in p53 but are increased in frequency in older women and to some degree in patients with ovarian cancer. Moreover, they harbor alterations in expression of PAX-2, ALDH1 (overexpression or underexpression), beta-catenin, and other genes altered in neoplasia.[156-158] They have no clinical significance in practice but are occasionally seen adjacent to rare beta-catenin positive endometrioid neoplasms of the tube (Brouwer J & Schmechler C, personal communication). They consist of type 1 SCOUTs, manifesting with ciliated differentiation (PAX2−/ALDH1−) and type 2 SCOUTs, which closely resemble endometrioid epithelium (PAX2−/ALDH1+/beta-catenin+) (Fig. 24.9A).[159] However, there are minimal atypia, preservation of pseudostratification, and a low MIB-1 index.

Serous Tubal Epithelial Proliferations/Lesions

Serous tubal epithelial proliferations, or serous tubal epithelial proliferations/lesions (STEPs) constitute clonally derived expansions or proliferations with p53 mutations.

Benign Serous Tubal Epithelial Proliferations/Lesions (p53 Signatures)

These constitute small minimally proliferative arrays of benign appearing epithelium with altered p53 (see Fig. 24.9B to D).[160] These small foci were initially designated as "p53 signatures."[161] p53 signatures are defined as benign appearing areas of tubal epithelium that show strong staining for p53 by immunohistochemistry. They have a secretory cell phenotype, a low proliferative index, and evidence of DNA damage by staining with γ-H2AX. In the study by Lee et al., about half of cases with STICs also have p53 signatures, and p53 mutations were identified in about half of p53 signatures by laser capture microdissection and PCR mutation analysis. Unlike STICs, p53 signatures

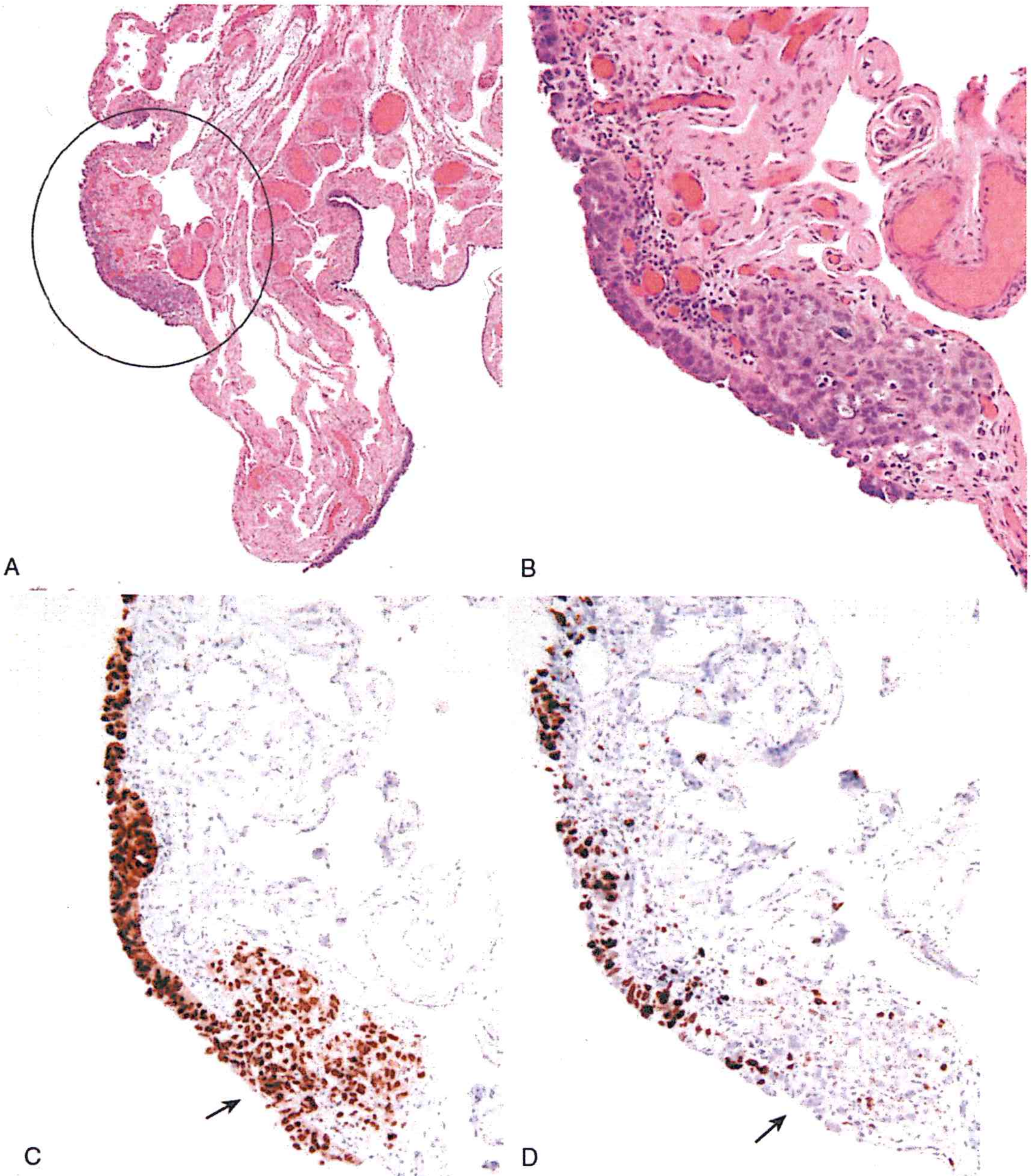

Fig. 24.8. A, Low power of a small focus of invasive carcinoma in a fimbrial plica. B, Same image at higher magnification. Following, p53 (C) and MIB-1 (D) immunostaining, note the lower than expected MIB-1 index in the invasive focus *(arrows)*.

are common in fallopian tubes regardless of *BRCA* mutation status.[162] Moreover, examination of prophylactically removed ovaries and fallopian tubes from *BRCA+* women showed that although p53 signatures are common in fallopian tubes, they are rare in ovarian epithelium.[162] One study addressed the epidemiologic risk factors for the development of p53 signatures, as compared with the known risk factors for ovarian carcinoma in this group.[163] However, although this entity may signify an early phase of serous cancer evolution, like many early lesions, it is common and clinically insignificant in the context of a salpingectomy, irrespective of its indications.

p53 signatures are morphologically indistinct or benign-appearing areas of tubal mucosa that are usually discovered incidentally when immunohistochemistry for p53 is performed. By definition, p53 signatures are at least 12 cells that are p53 positive by immunohistochemistry and have a low proliferative index (MIB-1 less than 10%) (see Fig. 24.9B).[109] Like their STIC counterparts, p53 signatures have a secretory cell phenotype. Signatures can be

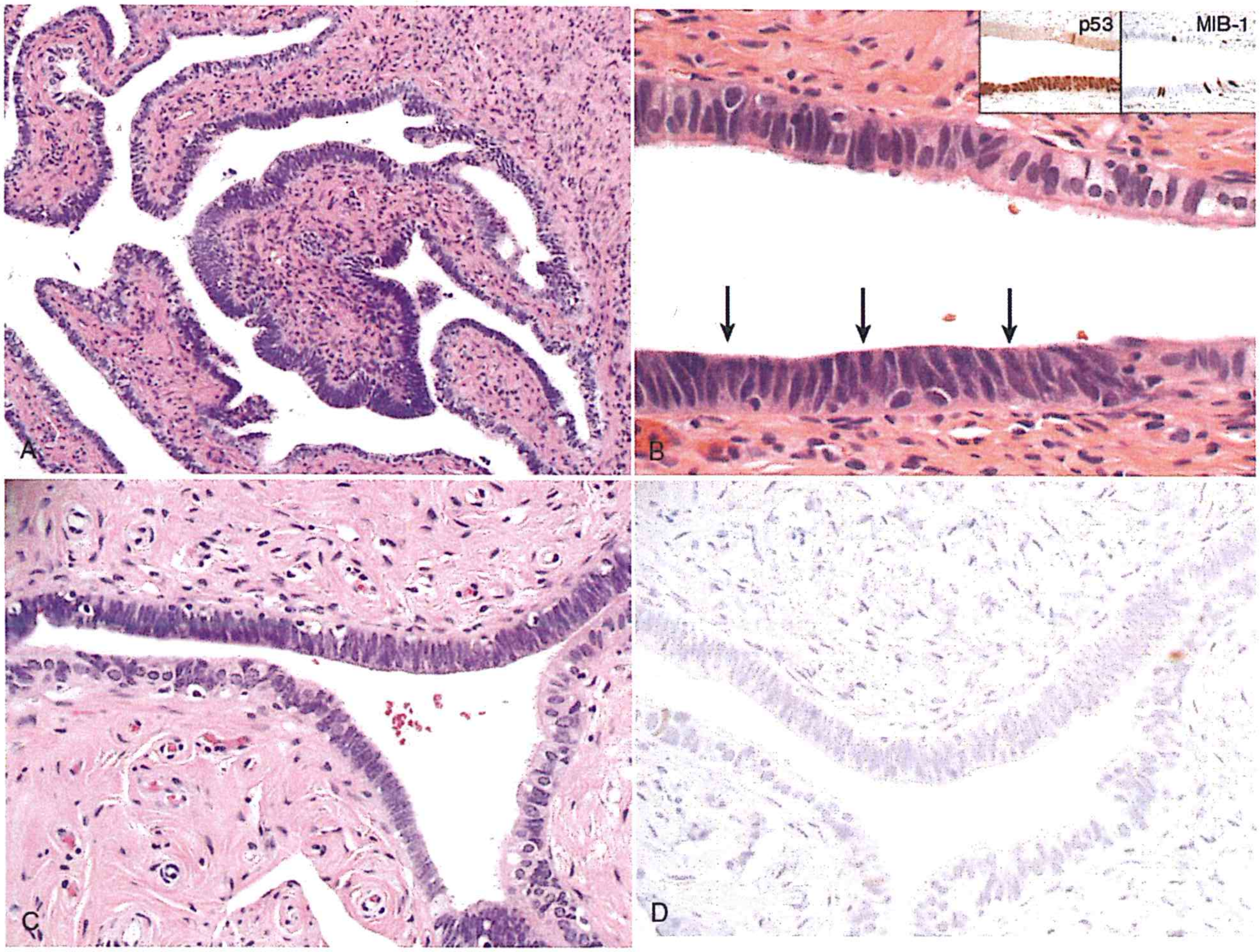

Fig. 24.9. The following must be excluded when making a diagnosis of serous tubal intraepithelial carcinoma (STIC) (additional examples can be seen in Chapter 21). A, Benign tubal epithelial proliferation (secretory/stem cell outgrowth [SCOUT]) with endometrioid differentiation. B, Benign serous tubal epithelial proliferation (p53 signature). Note the appearance of benign mucosa and low proliferative (MIB-1) index despite the strong staining for p53. C, Another p53 signature *(upper)*. D, It expresses no p53 protein, in keeping with a deletion (null) mutations.

continuous (an uninterrupted strip of p53-positive secretory cells) or discontinuous (p53-positive cells with some intervening ciliated cells).

Serous Tubal Epithelial Proliferations/Lesions of Uncertain Significance

The pathologist who scrutinizes prophylactic salpingo-oophorectomies will invariably encounter a process that cannot be readily classified. Jarboe et al. noted that, on rare occasion, atypical foci of p53-positive secretory cells can be identified, which exhibit features intermediate between p53 signatures and STICs.[164] These "atypical hyperplasias" demonstrate the following:

- Overall preservation of pseudostratification and epithelial polarity (Fig. 24.10)
- Inter-digitation with benign-appearing ciliated epithelium in some cases
- An increased MIB-1 index of 20% or more in at least a portion of the lesion
- Evidence of DNA damage manifested by immunopositivity for γ–H2AX

The authors originally designated these atypical p53-positive foci as tubal intraepithelial lesions in transition (TILT) for these. Vang et al. proposed the term *serous tubal intraepithelial lesion (STIL)*.[155] Lee et al. suggested the term *tubal epithelial atypia (TEA)*.[165] We would classify them as serous tubal epithelial proliferations or lesions of uncertain significance (STEP-US) for two reasons. First, distinguishing such proliferations of this type from STIC may not always be possible; interobserver reproducibility in separating the two is far from ideal, and in some instances one of these proliferations may be found in continuity with an invasive serous carcinoma. Second, despite the fact that they are presumably neoplastic, they confer minimal if any risk of subsequent pelvic serous carcinoma. In fact, the risk of a

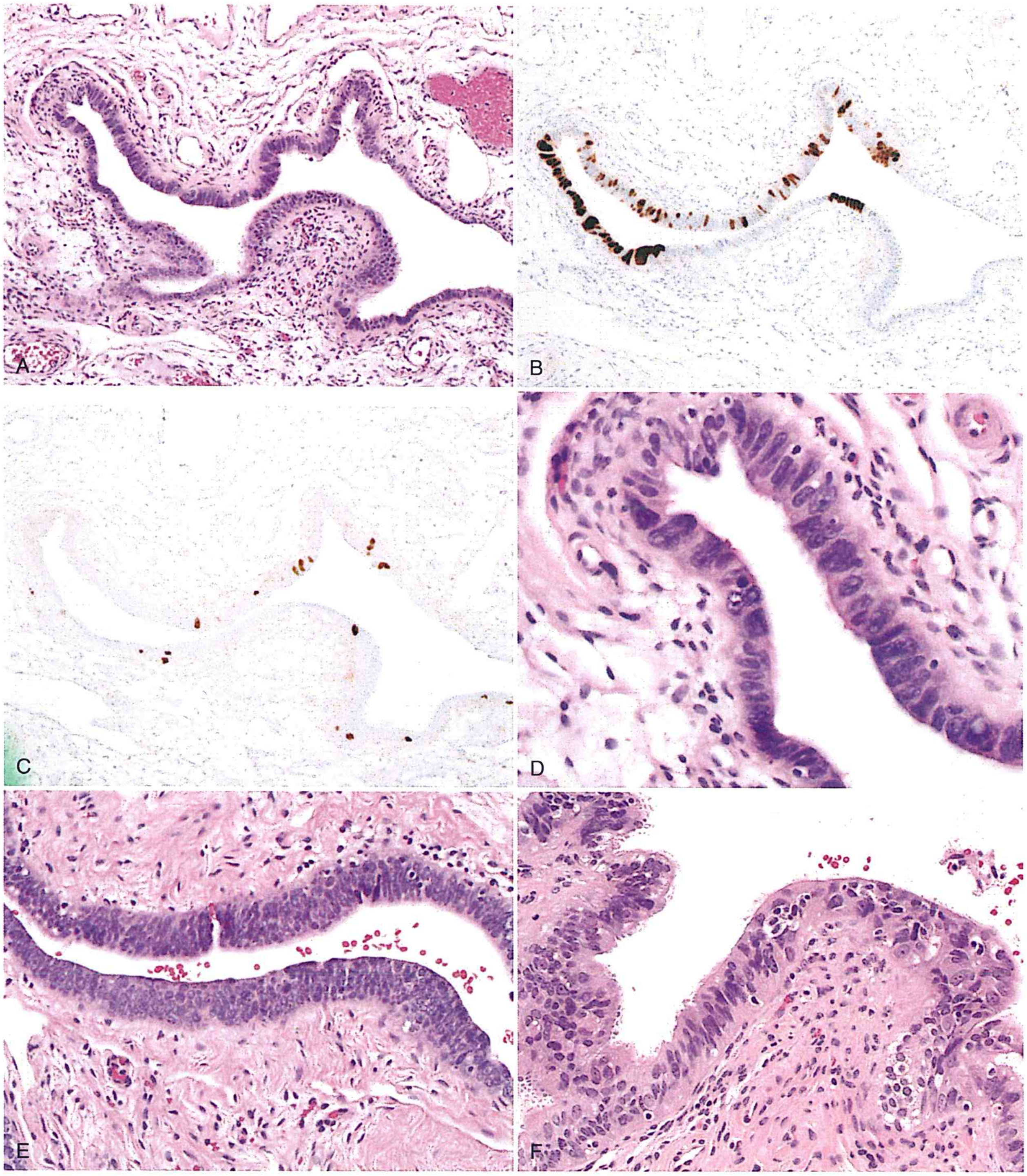

Fig. 24.10. Serous tubal epithelial proliferations/lesions of uncertain significance (STEP-USs). **A,** A proliferation with some nuclear enlargement and hyperchromasia. **B,** Note the focal strong p53 positivity. **C,** The MIB-1 index is low. **D,** Higher magnification of A showing the nuclear atypias. **E,** Another STEP-US. There is a monomorphic non-ciliated population with some nuclear enlargement; however, the lining is uniform with a cohesive cell population. **F,** Another STEP-US, most conspicuous on the right, with nuclear enlargement. This was strongly p53 positive; however, note the preservation of ciliated differentiation.

Table 24.3 Sample Terminology and Explanatory Notes for Incidentally Discovered Tubal Epithelial Abnormalities

Terms or Abbreviations Used	Diagnostic Term	Comment
SCOUT	Benign epithelial proliferation	None
p53 signature	Benign serous epithelial proliferation/lesion	None
STIL/TILT/TEA	STEP-US	This proliferation exhibits cellular atypia and is p53 positive, but cell polarity is maintained with a low proliferative index. It is not sufficient for a diagnosis of STIC.
STIC	STIC	This proliferation exhibits cellular atypia, evidence of a p53 mutation, loss of cell polarity, and an elevated proliferative index. When found in isolation, STICs carry an approximately 5% risk of subsequent pelvic HGSC based on the current literature.

HGSC, High grade serous carcinoma; *SCOUT*, secretory/stem cell outgrowth; *STEP-US*, serous tubal epithelial proliferation/lesion of uncertain significance; *STIC*, serous tubal intraepithelial carcinoma; *STIL*, serous tubal intraepithelial lesion; *TEA*, tubal epithelial atypia; *TILT*, tubal intraepithelial lesion in transition.

From Visvanathan K, Vang R, Shaw P, et al: Diagnosis of serous tubal intraepithelial carcinoma based on morphologic and immunohistochemical features: a reproducibility study. *Am J Surg Pathol* 35:1766-1775, 2011; Vang R, Visvanathan K, Gross A, et al: Validation of an algorithm for the diagnosis of serous tubal intraepithelial carcinoma. *Int J Gynecol Pathol* 31:243-253, 2012; Mehra K, Mehrad M, Ning G, et al: STICs, SCOUTs and p53 signatures; a new language for pelvic serous carcinogenesis. *Front Biosci (Elite Ed)* 3:625-634, 2011; Chen EY, Mehra K, Mehrad M, et al: Secretory cell outgrowth, PAX2 and serous carcinogenesis in the fallopian tube. *J Pathol* 222:110-116, 2010; Roh MH, Yassin Y, Miron A, et al: High-grade fimbrial-ovarian carcinomas are unified by altered p53, PTEN and PAX2 expression. *Mod Pathol* 23:1316-1324, 2010.

subsequent pelvic malignancy for isolated STIC is only 5%. *Uncertain significance* or a comparable term conveys this message.[166] Diagnostic terms and sample comments for these various tubal epithelial abnormalities are provided in Table 24.3.

Other Benign Tubal Abnormalities

On occasion, foci of benign tubal epithelium can be identified, which can raise suspicion for a neoplastic process. Tubal epithelium exhibiting architectural complexity with pseudostratification and occasional scattered atypical-appearing nuclei can be mistaken for neoplasia (see Chapter 21). In general, such a benign mimic will exhibit the presence of cilia diffusely, and overall polarity of the epithelium will be preserved. In especially challenging cases, immunostaining for p53 and MIB-1 can serve as a useful diagnostic adjunct. The reader should be cautioned, however, that positive staining for p53 and increased MIB-1 should never be used as a substitute for the appropriate morphologic features when assessing whether or not an STIC is present.

Clinical Impact

The thorough examination of prophylactic salpingo-oophorectomy specimens from women with *BRCA* mutations is critical both for the treatment and risk assessment of the individual patients and to gain a better understanding of the pathogenesis of pelvic cancer. These specimens offer a unique opportunity to observe preclinical lesions of the fallopian tube and ovary, knowledge that is vital to the development of future early detection programs. Brown et al. published a mathematical model of the natural history of serous ovarian carcinoma in order to estimate the "window of opportunity" in which intervention might be life-saving and to define more clearly a target for early detection.[167] This study used the literature on prophylactic salpingo-oophorectomy specimens from *BRCA-1* carriers to calculate the prevalence of early occult tumors and estimated the incidence of symptomatic carcinomas in the same population based on prospective studies. The model estimated that the duration of the window of opportunity for detecting early occult tumors was 4.3 years (CI = 2.6 to 6.9 years) and that most of the tumors in this population progress to an advanced stage by a mean of 0.8 years (CI = 0.4 to 1.9 years) before detection. The stage-specific survival data show that a 50% reduction in 5-year mortality could be achieved with an annual screen that detects a 4-mm tumor. The development of early detection modalities is critically dependent on our understanding of the natural development of these tumors, both in terms of size and rate of growth; proper evaluation of prophylactic specimens is the key to furthering this understanding.

The rate of progression of STICs to invasive serous carcinoma remains unknown, because biopsy and follow-up are not viable options in evaluation of the fallopian tube. A recent analysis of the literature reported that 4.5% of *BRCA+* women with isolated STICs developed primary peritoneal carcinoma within 72 months.[168] Other recent studies have supported the idea that the risk is low but not zero.[138,169,170] The role of adjuvant chemotherapy after a diagnosis of an incidental STIC remains uncertain; a recent meta-analysis reported that 16.4% of patients with a diagnosis of STIC in the literature received chemotherapy.[168] Most centers do not recommend chemotherapy if STIC is not accompanied by invasion or spread.

Early Ovarian Cancer

The frequency of early serous ovarian cancer is best put in perspective by the study by Bell and Scully, in which they identified 14 cases of early ovarian cancer, 10 of which were serous, from a large consultation file.[171] Precisely how many of these were bona fide serous carcinomas versus spread from an adjacent tubal primary is unknown, but the study underscores the rarity of early serous carcinomas.

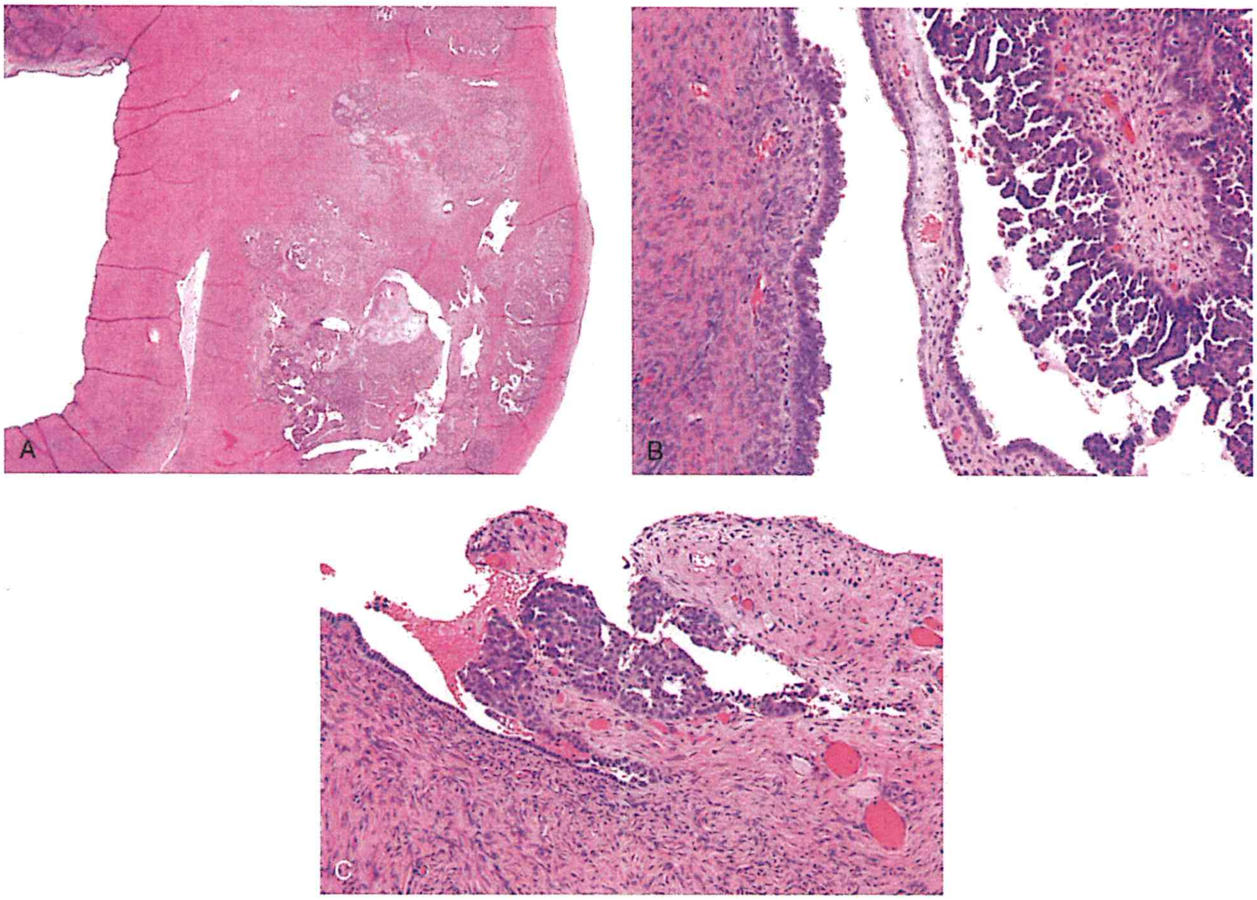

Fig. 24.11. A and B, Early ovarian carcinoma in a cyst from a *BRCA+* woman. C, Small surface implant of serous carcinoma on the ovary.

Occasional cases have been identified of apparent early primary ovarian malignancies in the *BRCA+* population, manifesting neoplastic cysts in the ovarian cortex. The criteria for diagnosis are similar to those for the fallopian tube (Fig. 24.11A and B).

More frequently, the pathologist may stumble on small foci of malignancy in the ovarian surface. Some of these likely signify metastases from the tube, but cases have been seen in which no tubal origin was identified (see Fig. 24.11C). Thus, the concept of surface ovarian carcinoma remains in play and emphasizes further the importance of carefully inspecting the ovarian surface in all prophylactic specimens.

KEY POINTS

- Risk-reducing surgery will reduce the death rate from ovarian cancer by over 80% in women with *BRCA-1* or *BRCA-2* mutations.
- From 5% to 10% of prophylactic surgical specimens from *BRCA+* women will harbor an early serous tubal intraepithelial carcinoma (STIC).
- Isolated STICs found in prophylactic salpingo-oophorectomies from *BRCA+* women have a low risk of a high-grade serous cancer outcome (≈5%). The role of chemotherapy in these patients is controversial, and most institutions do not treat women with STIC alone.
- Benign tubal mucosa can appear atypical, with variations in nuclear size and even nuclear molding. Critical features of STIC in hematoxylin and eosin (H&E)-stained sections are loss of polarity, rounded rather than elongated cell shape, intraepithelial fractures, and exfoliation. The latter two features will invariably be accompanied by irregular epithelial outlines with stratification and help in distinguishing STIC from serous tubal epithelial proliferations/lesions of uncertain significance (STEP-US).

Please see the Appendix for suggested ICD-10 codes.

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